

# Genetic Variation in Seedlings of *Picea mariana* (Mill.) BSP.

## I. Correlation with Ecological Factors

By E. K. MORGENSTERN<sup>1)</sup>

(Received for publication May 10, 1968)

### Introduction

*Picea mariana*, black spruce, is an interesting species of the boreal forests of North America. Its early evolution appears to be linked to the expansion of boreal elements from Alaska to the Atlantic Coast, following mountain building and development of continental climates during the cool Pliocene Epoch (HEER 1868, DORF 1960). The fossil record is younger and dates back to the Pleistocene Epoch, showing that the species was then widely distributed in central and eastern regions (LAMORTE 1952, BRYAN 1954). The present range extends from Alaska southward to the Lake States, southern Ontario, New York and Pennsylvania, and eastward to the Atlantic (HEINSELMAN 1957). Throughout its boreal range black spruce is one of the most common species. It possesses great reproductive capacity by seed and layering, regenerates well after fires, is adaptable to various soils and local climates, and is found in association with all other boreal species. Pure stands maintain themselves only on shallow, poorly drained and cold soils where competitors are lacking (MILLAR 1936, LINTEAU 1955, ROWE 1959, LOUCKS 1959-60, HORTON and LEES 1961, HEINSELMAN 1963, DAMMAN 1964, BAKUZIS and HANSEN 1965, VINCENT 1965). The large volumes of black spruce available and the high quality of its wood make this Canada's most important pulpwood species (BESLEY 1959, BASHAM and MORAWSKI 1964).

In recent years increased management of boreal forests has resulted in more artificial regeneration, a development which makes genetic studies more urgent. Particularly important at this stage are studies of geographic variation to ensure that only well adapted populations are being established, and to test populations for selection and breeding. Several field experiment with black spruce were established by HOLST (1962), but results from these are not yet available. This paper presents the findings of more recent greenhouse and nursery experiments. In Part I an attempt is made to correlate physiological and morphological variation with ecological factors. Ecological factors, in controlling natural selection, are expected to influence some of the responses of this species to its environment. This influence is traditionally measured by correlation and regression but recently new methods have become available. One of these, principal component analysis, was employed here and simplified the recognition of the most important factors. In Part II, a companion paper, the degree of subdivision of the species resulting from the combined effect of natural selection, mutation, recombination, migration and isolation is determined by a hierarchical analysis of variance showing differentiation at the level of populations, subpopulations, and half-sib families. Information on the variation of black

spruce obtained in this way is important for the planning of further studies and basic for the development of breeding programs.

### Objectives

The objectives of this study were to test two hypotheses concerning the genetic variation pattern of black spruce:

1. Genetic differentiation is mainly the result of natural selection by continuous ecological factors such as regional temperatures and day length, leading to clinal variation.
2. Genetic differentiation is subject to discrete factors as well, such as local climate and soil moisture. The variation pattern is therefore clinal and ecotypic.

### Methods

#### Seed Collection

In September and October, 1963 seed was collected in an area between 42 and 60 degrees of N. latitude and 77 and 112 degrees of W. longitude from 9 forest regions, 24 stands, and 148 single trees. Stands within regions were selected on the basis of 5 soil moisture regimes ranging from moderately dry to wet (Fig. 1-1, Table 1-1). Collections in Ontario included 21 of the 24 stands and sampled that part of each region where the greatest range of soil moisture regimes and local climates could be found. By concentrating each regional sample within a 20-kilometer radius, effects of clinal variation were greatly reduced, making it possible in the analysis

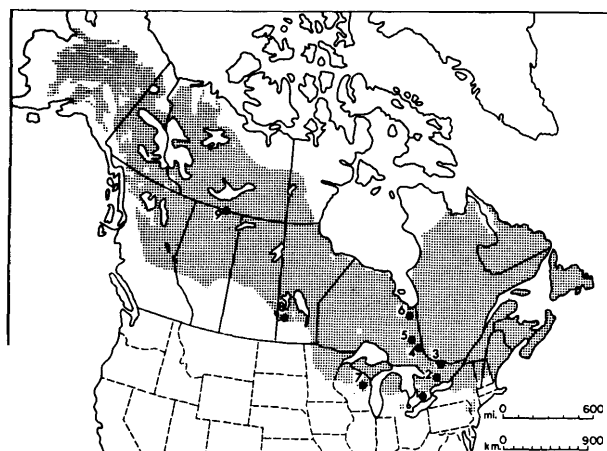


Figure 1-1. — Map of North America showing the range of *Picea mariana*, black spruce (U.S. Dept. of Agriculture 1965), and the origin of the 9 seed populations that formed the basis of this study.

to attribute within-region variability largely to ecotypic differentiation. But this procedure may prohibit the extension of inferences to other regions (per. comm. from Dr. D. M. BROWN, Biometrics Research Services, Department of Fisheries and Forestry).

Collection in Wisconsin was arranged by Dr. H. NIENSTAEDT, U. S. Department of Agriculture; in Manitoba by Mr. C. C. THOMPSON, Department Fisheries and Forestry; and in the Northwest Territories by Mr. R. H. KENDALL, Department of Indian Affairs and Northern Development.

In order to obtain uniform seed for physiological experiments, collection was generally limited to the 1963 crop and made in a south-to-north sequence, in conformity with the progression of seed maturation (Ontario Dept. of Lands and Forests 1963). All seed lots were extracted manually to avoid selection of different

<sup>1)</sup> Research Scientist, Department of Fisheries and Forestry, Petawawa Forest Experiment Station, Chalk River, Ontario, Canada. This paper is based upon a dissertation for the degree of Dr. rer. nat. submitted in 1966 to the Mathematisch-Naturwissenschaftliche Fakultät, Universität Hamburg, Germany. The larger part of the experimental work was performed at Institut für Forstgenetik und Forstpflanzenzüchtung, Bundesforschungsanstalt für Forst- und Holzwirtschaft, Schmalenbeck, Germany.

Table I-1. — Origin of seed. Populations correspond to regions, subpopulations to stands. Regions are based upon Rowa (1959) and U.S. Dept. of Agriculture (1948). The soil moisture regimes recognized were: 1 — moderately dry; 2 — fresh; 3 — very fresh; 4 — moist; 5 — wet.

| Population |                            |     |                       | Subpopulation |              |             |               |                     |                                     | Single trees                                    |        |
|------------|----------------------------|-----|-----------------------|---------------|--------------|-------------|---------------|---------------------|-------------------------------------|---|--------|
| No.        | Forest Region              | No. | Province or State     | Latitude °N   | Longitude °W | Elevation m | Soil Moisture | Land type           | Stand type                          | Ground Vegetation type                          | Number |
| 1          | Deciduous                  | 1   | Ontario               | 42°42'        | 80°20'       | 200         | 4             | Swamp lowland       | Thuja occidentalis<br>Picea mariana | Hypnum spp.<br>Cornus canadensis                | 9      |
| 2          | Great Lakes - St. Lawrence | 2   | Ontario               | 44°10'        | 78°41'       | 250         | 4             | Swamp lowland       | Picea mariana<br>Picea glauca       | Hypnum spp.<br>Linnaea borealis                 | 6      |
|            |                            | 3   | Ontario               | 44°09'        | 78°44'       | 250         | 4             | Swamp lowland       | Thuja occidentalis<br>Picea mariana | Hypnum spp.<br>Graminae                         | 2      |
|            |                            | 4   | Ontario               | 44°20'        | 78°32'       | 250         | 4             | Swamp lowland       | Thuja occidentalis<br>Picea mariana | Hypnum spp.<br>Coptis groenlandica              | 6      |
| 3          | Great Lakes - St. Lawrence | 5   | Ontario               | 45°58'        | 77°25'       | 200         | 1             | Sandy hills         | Picea mariana<br>Picea glauca       | Vaccinium spp.<br>Comptonia peregrina           | 6      |
|            |                            | 6   | Ontario               | 45°57'        | 77°24'       | 200         | 2             | Bog - upland border | Picea mariana<br>Larix laricina     | Comptonia peregrina<br>Sphagnum spp.            | 6      |
|            |                            | 7   | Ontario               | 45°59'        | 77°28'       | 200         | 5             | Creek valley        | Picea mariana<br>Larix laricina     | Sphagnum spp.<br>Hypnum spp.                    | 6      |
|            |                            | 8   | Ontario               | 46°02'        | 77°22'       | 200         | 4             | Swamp lowland       | Picea mariana<br>Larix laricina     | Alnus rugosa<br>Sphagnum spp.                   | 6      |
|            |                            | 9   | Ontario               | 45°31'        | 77°10'       | 200         | 5             | Bog lowland         | Picea mariana                       | Ledum groenlandicum<br>Sphagnum spp.            | 6      |
| 4          | Boreal                     | 10  | Ontario               | 48°00'        | 80°08'       | 300         | 5             | Bog lowland         | Picea mariana                       | Sphagnum spp.<br>Hypnum spp.                    | 6      |
|            |                            | 11  | Ontario               | 48°00'        | 80°08'       | 300         | 5             | Bog lowland         | Picea mariana                       | Sphagnum spp.<br>Gaultheria hispidula           | 7      |
|            |                            | 12  | Ontario               | 47°59'        | 80°09'       | 300         | 1             | Sandy hills         | Pinus banksiana<br>Picea mariana    | Vaccinium spp.<br>Cornus canadensis             | 6      |
|            |                            | 13  | Ontario               | 47°59'        | 80°09'       | 300         | 2             | Sandy plain         | Pinus banksiana<br>Picea mariana    | Pleurozium schreberi<br>Ledum groenlandicum     | 6      |
|            |                            | 14  | Ontario               | 47°59'        | 80°09'       | 300         | 3             | Bog - upland border | Picea mariana<br>Pinus banksiana    | Pleurozium schreberi<br>Sphagnum spp.           | 6      |
| 5          | Boreal                     | 15  | Ontario               | 48°30'        | 80°50'       | 300         | 1             | Sandy plain         | Picea mariana<br>Pinus banksiana    | Vaccinium myrtilloides<br>Oryzopsis asperifolia | 6      |
|            |                            | 16  | Ontario               | 48°30'        | 80°50'       | 300         | 2             | Sandy slope         | Picea mariana<br>Betula papyrifera  | Pleurozium schreberi<br>Ledum groenlandicum     | 6      |
|            |                            | 17  | Ontario               | 48°55'        | 80°59'       | 300         | 3             | Loamy hills         | Abies balsamea<br>Picea mariana     | Alnus rugosa<br>Cornus canadensis               | 6      |
|            |                            | 18  | Ontario               | 48°58'        | 81°00'       | 300         | 4             | Bog lowland         | Picea mariana                       | Sphagnum spp.<br>Alnus rugosa                   | 6      |
|            |                            | 19  | Ontario               | 48°53'        | 80°54'       | 300         | 5             | Bog lowland         | Picea mariana                       | Ledum groenlandicum<br>Sphagnum spp.            | 6      |
| 6          | Boreal                     | 20  | Ontario               | 51°16'        | 80°46'       | 50          | 4             | River shore         | Picea mariana<br>Larix laricina     | Sphagnum spp.<br>Calamagrostis sp.              | 6      |
|            |                            | 21  | Ontario               | 51°06'        | 80°52'       | 50          | 3             | River terrace       | Picea mariana                       | Hypnum spp.<br>Pleurozium schreberi             | 6      |
| 7          | Northern                   | 22  | Wisconsin             | 45°25'        | 88°40'       | 400         | 4             | Swamp lowland       | Picea mariana<br>Abies balsamea     | Not determined                                  | 9      |
| 8          | Boreal                     | 23  | Manitoba              | 51°53'        | 100°51'      | 800         | 3             | Moraine             | Picea mariana<br>Picea glauca       | Pleurozium schreberi<br>Hylocomium splendens    | 7      |
| 9          | Boreal                     | 24  | Northwest Territories | 60°03'        | 112°46'      | 250         | 2             | Not known           | Picea mariana<br>Larix laricina     | Not determined                                  | 6      |

portions of the seed populations by sieves and air currents (MacGILLIVRAY 1956).

### Experimental Program

Four experiments were conducted, each investigating a different stage in seedling development. Following an estimate of the total amount of seed available per single-tree collection and the weighing of 1000 seeds from each, the seed material was allotted to the four experiments. This involved considerations of greenhouse and nursery space and the particular purpose of each experiment. Choice of single-tree seedlots was at random with the restriction that large lots contained enough seed for all experiments but small lots for only one or two.

Experiment No. 1: — Germination and early development in a greenhouse at the Petawawa Forest Experiment Station, latitude 46° N, longitude 77° W.

Seed from all 9 regions was included in this experiment: 115 single-tree collections from the 6 regions in Ontario plus 3 stand collections, one from each of the 3 western regions; each stand collection consisted of equal amounts

of seed from 4 trees. This experiment was begun in December, 1963. Empty seeds were removed by flotation in absolute ethyl alcohol. From the full seeds, lots of 50 each were stratified by placing them on moist fungicide-treated absorbent paper in Petri dishes at 20° C for 3 weeks. A randomized block layout with 8 replications was generally used, containing 118 plots in the first and fewer plots in the last replications as a result of the small seedlots from southern regions. Following stratification the seeds in each dish were sown into a sand-humus mixture in a plastic pot and placed into a greenhouse. Here each pot represented a plot in a randomized block layout, with blocks arranged to reduce the effects of variation in light and temperature. Soil temperatures at seed level averaged 10° C during nights and 16° C at day time. Illumination with artificial light of 4500 lux intensity extended the photoperiod to 20 hours. Germination began 10 days after sowing and was recorded every second day until the 24th day, and again on the 31st day when survival of the germinant seedlings was also noted. Hypocotyl length and cotyledon number were subsequently determined. The long-day treatment

was concluded in August and the experiment terminated with a height measurement in October, 1964.

**Experiment No. 2:** — Survival of six-weeks-old seedlings under continuous drought conditions in a greenhouse at Institut für Forstgenetik in Schmalenbeck, Germany, latitude 54° N, longitude 10° E.

This experiment was concerned with the central regions only where the species occupies a wide range of sites. Thirty-seven single-tree progenies from 5 moisture regimes in regions B.4 and B.7 (Table I-I), were germinated in July, 1964. They were then planted at a regular spacing into trays according to a standard randomized block design with 6 replications, 20 seedlings constituting a plot, with 37 plots per block. After hardening for one month in the open, 4 replications were taken back into the greenhouse and kept dry to induce drought conditions. Survival was noted every second day in the initial and every day in the final stages of the test until all plants had died. The trays were weighed on the same days. The remaining 2 replications, kept outside, were then harvested, root and shoot lengths measured, dried at 104° C and weighed. The data obtained from these 2 replications were used for correlation with this and the following experiment.

**Experiment No. 3:** — Dry-matter production of first-year seedlings under intermittent drought conditions in a greenhouse at Institut für Forstgenetik in Schmalenbeck.

Plants of the same origin as in Experiment No. 2 with 4 replications, 18 plants per plot, and 37 plots per block were tested in a randomized block design during the period October 15, 1964 to March 30, 1965. Three drought periods were induced by omitting watering until needle mortality occurred, each drought treatment taking about six weeks for completion. The trays were then re-watered by inserting them into plastic tubs to attain a uniform moisture content approximating field capacity (LUTZ and CHANDLER 1946, p. 316). Greenhouse temperatures ranged from 10° to 30° C. Artificial illumination at an intensity of 450 lux during nights was necessary to weaken the tendency for terminal bud formation and growth cessation. After each drought period a measurement of shoot lengths was made and the number of plants with terminal buds was counted. Following the last drought period, shoot and root lengths, and dry weights were determined.

**Experiment No. 4:** — Phenology and growth of first and second-year seedlings in the nursery at Institut für Forstgenetik in Schmalenbeck.

In May, 1964 seed from 121 single trees in all 9 regions was sown on needle-litter seedbeds in a modified lattice design with 4 replications. Plots consisted of plant rows 1.2 meters long, 11 centimeters apart, and were numbered in sequence to avoid bias owing to original collection numbers. Plots were not arranged by rows and columns but side by side; a complete replication thus required about 13.2 meters of seedbed length. Sowing was aimed at keeping density low to avoid competition over the two-year testing period. Within each plot, about 25–400 seeds were sown and 20 seedlings were marked at random with plastic rings for observation and measurement. Germination and growth were satisfactory. Growth initiation occurred much earlier and cessation later than in native habitats, in response to the longer growing season of North Germany. Over the range of provenances this required long observation periods. In August, 1964 the percentage of seedlings with proleptic shoots was determined, including those with shoot extension from both terminal and lateral buds. From August 29th to November 25th, 1964 the number of seed-

lings with visible terminal buds was counted at two-day intervals as an indicator of the cessation of height growth. From April 21st, 1965 to May 29th, 1965 the number of seedlings with bursting buds was counted at two-day intervals to measure initiation of height growth, and from July 20th to November 15th the number of terminal buds was again recorded as in 1964. Height measurements concluded this experiment.

#### Analysis

Experimental results were computed on the basis of plot and progeny means using methods that would lead to approximately normal distributions. Germination and survival rates were calculated as the rate index devised by BARTLETT (1937) and percentage values transformed by arcsin. A test of normality (SNEDECOR 1956, p. 201) applied in one doubtful case showed that after transformation there was no detectable departure from normality in the distribution. For the calculation of missing values a randomized block layout was assumed for all experiments and the procedure then followed was that of STEEL and TORRIE (1960).

For correlation with ecological factors, climatic data were obtained from the Climatic Atlas of the United States (VISHER 1954) and the Atlas of Canada (Dept. of Mines and Technical Surveys 1957). Day length values were taken from WITHROW (1959). These data, and soil moisture regime, although only approximately normal, were not transformed. The results thus obtained are approximations and apply particularly to the sample included in this study.

The data were analysed by correlation, simple and multiple regression, and principal component analyses (MORGENSTERN 1966). The correlation and principal component analyses summarized the data in the simplest manner and only these will therefore be given in this paper. Correlation analysis is a standard procedure and requires no explanation. The mathematical basis of principal component analysis is given by HOTELLING (1936), HARMAN (1960), KENDALL (1957), and SEAL (1964) while biological applications with detailed explanations of the procedure are offered by FRUCHTER (1954), KRAUS and CHOI (1958), WILLIAMSON (1961), CASSIE (1963), PEARCE (1965), and VAN GROENEWOUD (1965). Only a brief non-mathematical outline will be given here to explain the terms used in the discussion.

The problem to be dealt with is as follows: A set of variates has been observed on each of a number of trees and the matrix of correlation coefficients between all variates has been calculated. In contrast to regression, all variates are considered equal and no distinction between dependent and independent variates is made. The analysis then aims to examine the relationship among the variates, to look for components in order to reduce the dimension of variation, and, if possible, to interpret the components found in terms of real physical or biological factors (KENDALL 1957).

The computations begin with the formation of the characteristic equation of the square matrix of correlation coefficients by subtracting from the diagonal, i. e. the ones, a value designated as  $\lambda$ , which is chosen so that the determinant of the new equation is equal to zero. The various  $\lambda$ 's are called eigenvalues, characteristic roots, or latent roots. The numerical solution of the equation for these is much facilitated by the use of a computer. These eigenvalues are found in descending order of magnitude,  $\lambda_1 > \lambda_2 > \lambda_3$ , etc. The sum of all eigenvalues is equal to the number of varieties in the correlation matrix. Each eigenvalue has an eigenvector associated with it.

What has been done mathematically is to find the linear

function which has the largest variance, then the linear function orthogonal i.e. uncorrelated with the first, again with the largest variance, and so on. In geometrical terms, axes are fitted to a series of points in multidimensional space, first finding the longest axis of the appropriate ellipsoid, then the longest axis which is at right angles to the first, etc. The length of each axis is indicated by its eigenvalue and the direction of that axis by the corresponding eigenvector. Eigenvalues indicate the percentage of total variance accounted for by individual axes.

In summary, what the analysis accomplishes is a transformation of the old correlated variates into new variates or components (i.e. linear combinations of old variates) which are uncorrelated, and independent, if based on an underlying normal multivariate distribution. This usually results in a great reduction in the dimension of variation. For example, a set of 10 variates requiring a 10-dimensional space initially may be reduced to 2-4 dimensions accounting for the most essential part of total variation. But whether the axes representing the components are merely convenient mathematical artefacts or have a physical or biological meaning, remains to be seen. The investigator's familiarity with his subject is essential for an interpretation of the components (KENDALL 1957, WALLIS 1965).

In this study the analysis was performed on the TR4 computer of the Universität Hamburg, Germany. The program was written in the Algol language and utilized the JACOBI method of matrix diagonalization (WHITE 1958). The analysis included several features of factor analysis by printing factor loadings (WALLIS 1965) instead of eigenvector coefficients and by continuing with a varimax rotation (HARMAN 1960) after a standard principal component analysis. Significantly, the interpretation of the components before and after rotation was the same, and their order was also the same except in one case. But rotation had simplified the factor loadings by concentrating loadings more strongly on some variables, thus making the interpretation easier. The results from the rotated axes will therefore be given in this paper. — Although principal component and factor analysis are based upon different mathematical models, they can be usefully applied in succession (KENDALL 1957, SEAL 1964), as in this case. For simplicity the present analysis will be termed principal component analysis which conforms with the description of the computer program.

## Results and Discussion

### Experiment No. 1: — Germination and Early Development

The correlation analysis (Table I-2) showed that both germination rate and germination percent increased with

seed weight and latitude but decreased with increasing soil moisture regime of the place of seed origin. Survival percent increased with latitude and decreased with soil moisture. Cotyledon number and hypocotyl length increased with seed weight and decreased with latitude. The height of the ten-months-old seedlings decreased with a higher germination percent.

Although many of the correlations are temptingly suggestive and known from other experiments (WRIGHT and BULL 1962, BURGAR 1964, BURLEY 1964, ANDERSSON 1965), no cause-and-effect relationship can be derived from them. Because correlation is strictly an association analysis (STEEL and TORRIE 1960), conclusions will be based upon the principal component analysis which is more reliable.

The results of the principal component analysis are shown in Table I-3. Here results were grouped on the basis of the principal axes about which the variance is maximum, and the eigenvalues refer to the sum of squares of loadings for each axis. The three highest loadings have been underlined and will be used to identify the component. Components will be termed factors in recognition of the purposes of this study.

The highest loadings for the first four axes were as follows:

Axis 1: cotyledon number, hypocotyl length, seed weight;  
Axis 2: germination rate, survival percent, soil moisture regime;

Axis 3: hypocotyl length, latitude, longitude;

Axis 4: germination rate, germination percent, ten-months height.

It is clear that Axis 1 involved a morphological relationship, namely size, and the underlying factor was designated as seed size. The importance of seed size and weight for germination and early growth is known at least since Professor BÜHLER's studies in 1891 (BALDWIN 1942). That the number of cotyledons and hypocotyl length depend upon seed size or weight has already been shown by BARTELS (1956), MACGILLIVRAY (1956), and YEATMAN (1966a, 1966b).

Axis 2 involved mainly physiological relationships and the underlying factor was termed vigour. The negative loading for soil moisture regime confirmed the dependence of germination rate and survival percent upon soil moisture: germination rate and survival percent decreased with increasing soil moisture, measured by soil moisture regime of the locality of origin. This result parallels the findings of MACLEAN and BEDELL (1955) who have shown that in natural stands of black spruce dominant heights decrease from the moist through the wet to the very wet sites. Similarly, HORTON and LEES (1961) and LOWRY (1964) indicated that heights decrease from fresh to wet sites, in consequence of higher water tables and thicker humus

Table 1-2. — Correlation coefficients of Experiment No. 1 based upon pooled data from 115 single-tree progenies and 3 stands in Populations 1 to 9.)

| Variables                           | x <sub>1</sub> | x <sub>2</sub> | x <sub>3</sub> | x <sub>4</sub> | x <sub>5</sub> | x <sub>6</sub> | x <sub>7</sub> | x <sub>8</sub> | x <sub>9</sub> | x <sub>10</sub> |
|-------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|
| x <sub>1</sub> Germination rate     | +1.00          | +0.59***       | +0.53***       | +0.01          | +0.31**        | -0.15          | +0.33***       | -0.27**        | +0.25*         | +0.07           |
| x <sub>2</sub> Germination percent  |                | +1.00          | +0.13          | +0.01          | +0.27**        | —              | +0.26**        | -0.19*         | +0.19*         | +0.00           |
| x <sub>3</sub> Survival percent     |                |                | +1.00          | -0.24**        | -0.11          | +0.12          | -0.05          | -0.27**        | +0.21*         | +0.07           |
| x <sub>4</sub> Cotyledon number     |                |                |                | +1.00          | +0.62***       | 4.08           | +0.62***       | 4.01           | -0.44***       | -0.25**         |
| x <sub>5</sub> Hypocotyl length     |                |                |                |                | +1.00          | -0.01          | +0.63***       | -0.01          | -0.52***       | -0.27**         |
| x <sub>6</sub> Ten-months height    |                |                |                |                |                | +1.00          | -0.15          | +0.04          | -0.08          | +0.08           |
| x <sub>7</sub> Seed weight          |                |                |                |                |                |                | +1.00          | -0.09          | -0.25**        | -0.12           |
| x <sub>8</sub> Soil moisture regime |                |                |                |                |                |                |                | +1.00          | -4.14          | -0.09           |
| x <sub>9</sub> Latitude N           |                |                |                |                |                |                |                |                | +1.00          | +0.62***        |
| x <sub>10</sub> Longitude W         |                |                |                |                |                |                |                |                |                | +1.00           |

Significance levels in all correlation tables: \*, 5%; \*\*, 1%; —, 0.1%.

Note 1-3: — Results of the principal component analysis of Experiment No. 1. The three highest loadings for each axis have been marked to identify the underlying factor. Axis 5, with an eigenvalue smaller than 1, was not printed after varimax rotation and is here given from the earlier standard analysis.

| Axis                      | 1         | 2      | 3                 | 4           | 5             |
|---------------------------|-----------|--------|-------------------|-------------|---------------|
| Eigenvalue                | 2.45      | 1.93   | 1.60              | 1.55        | 0.84          |
| Percent of total variance | 25        | 19     | 16                | 15          | 8             |
| Variables                 | Loadings  |        |                   |             |               |
| Germination rate          | +0.23     | +0.79  | +0.11             | —0.36       | +0.25         |
| Germination percent       | +0.17     | +0.40  | +0.07             | —0.74       | +0.12         |
| Survival percent          | —0.23     | +0.85  | —0.05             | +0.13       | +0.13         |
| Cotyledon number          | +0.85     | —0.16  | —0.15             | +0.02       | —0.14         |
| Hypocotyl length          | +0.84     | +0.13  | —0.27             | —0.07       | +0.15         |
| Ten-months height         | —0.00     | +0.14  | +0.04             | +0.88       | +0.20         |
| Seed weight               | +0.85     | +0.14  | +0.01             | —0.16       | +0.03         |
| Soil moisture regime      | —0.06     | —0.54  | —0.13             | +0.01       | +0.81         |
| Latitude N                | —0.40     | +0.22  | +0.77             | —0.19       | +0.05         |
| Longitude W               | —0.09     | +0.04  | +0.93             | +0.10       | +0.05         |
| Factor interpretation     | Seed size | Vigour | Geographic origin | Competition | Soil moisture |

layers on the moist and wet sites. The studies of WEETMAN (1962) suggest that nitrogen is more easily available on the drier sites, largely as a result of more favourable temperatures for humus decomposition. YOUNGBERG (1952) has shown that soil fertility influences seed composition, i.e. the content of nitrogen, phosphorus, potassium and magnesium, and that large differences in germination percent exist between seed grown on fertile and infertile soils. Other causes of variation such as seed age and maturity (LANGNER 1960, SCHELL 1960, ROHMEDEYER 1962) were eliminated or their magnitude reduced by the collection procedure and stratification applied in this study. Beyond these environmental causes for differences in early vigour, there are those of genetic origin, particularly inbreeding (RIGHTER 1945).

Next, axis 3 was mainly characterized by large loadings for the variables longitude and latitude and it was therefore most closely related to the factor geographic origin. Hypocotyl length and cotyledon number were also linked to this axis, and negative values indicated that they decreased with higher latitudes, but the loadings were too small to be of much significance.

Finally, axis 4 carried large loadings for the variables germination percent and ten-months height. Here the tallest plots were those with the smallest number of ger-

minating seeds and the underlying factor was therefore designated as competition.

All four axes together accounted for 75 percent of the total variance. A fifth axis, calculated prior to rotation, was related to soil moisture of the place of origin and added 8 percent to total variance.

#### Experiment No. 2: — Survival under Continuous Drought Conditions

The correlation analysis (Table I-4) showed that survival was significantly related to lengths and dry weights of roots and shoots, to seed weight, and to water loss of the trays in which the plants were growing.

The principal component analysis (Table I-5) revealed that the first five axes were most closely related to factors interpreted here as water stress, plant size, relative root size, seed size and soil moisture of the place of seed origin.

The factor water stress ranked first in importance as shown by the large loadings for survival.

Plant size and seed size were secondary factors. Although the axes with which they were associated accounted for nearly the same amount of total variance as the first factor, loadings for the survival criteria were smaller. Observations and the data indicated that plant size played a role because larger plants were capable of drawing water from

Table I-4. — Correlation coefficients of Experiment No. 2 based upon 37 single-tree progenies from each of 5 soil moisture regimes in Populations 4 and 5.

| Variables                                   | x <sub>1</sub> | x <sub>2</sub> | x <sub>3</sub> | x <sub>4</sub> | x <sub>5</sub> | x <sub>6</sub> | x <sub>7</sub> | x <sub>8</sub> | x <sub>9</sub> | x <sub>10</sub> |
|---|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|
| x <sub>1</sub> Survival: Bartlett Index     | +1.00          | +0.94***       | +0.71***       | +0.68***       | +0.57***       | +0.61***       | —0.13          | +0.58***       | —0.14          | +0.91***        |
| x <sub>2</sub> Survival: days to 50 percent |                | +1.00          | +0.70***       | +0.65***       | +0.57***       | +0.53***       | —0.15          | +0.49**        | —0.11          | +0.98***        |
| x <sub>3</sub> Root dry weight              |                |                | +1.00          | +0.90***       | +0.80***       | +0.79***       | —0.28          | +0.69***       | —0.10          | +0.70***        |
| x <sub>4</sub> Shoot dry weight             |                |                |                | +1.00          | +0.62***       | +0.83***       | +0.15          | +0.72***       | —0.11          | +0.64***        |
| x <sub>5</sub> Root length                  |                |                |                |                | +1.00          | +0.56***       | —0.46**        | +0.42**        | —0.21          | +0.57***        |
| x <sub>6</sub> Shoot length                 |                |                |                |                |                | +1.00          | +0.00          | +0.56***       | —0.12          | +0.54***        |
| x <sub>7</sub> Shoot-root ratio, dry-weight |                |                |                |                |                |                | +1.00          | —0.01          | —0.04          | —0.15           |
| x <sub>8</sub> Seed weight                  |                |                |                |                |                |                |                | +1.00          | —0.03          | +0.47**         |
| x <sub>9</sub> Soil moisture regime         |                |                |                |                |                |                |                |                | +1.00          | —0.06           |
| x <sub>10</sub> Percent water loss of trays |                |                |                |                |                |                |                |                |                | +1.00           |

Table I-5. — Results of the principal component analysis of Experiment No. 2.

| Axis                         | 1            | 2                   | 3                  | 4         | 5             |
|------------------------------|--------------|---------------------|--------------------|-----------|---------------|
| Eigenvalue                   | 3.14         | 2.87                | 1.37               | 1.09      | 1.05          |
| Percent of total variance    | 31           | 29                  | 14                 | 11        | 10            |
| Variables                    | Loadings     |                     |                    |           |               |
| Survival: Bartlett Index     | −0.87        | +0.33               | −0.07              | +0.24     | −0.09         |
| Survival: days to 50 percent | −0.94        | +0.29               | −0.09              | +0.12     | −0.05         |
| Root dry weight              | −0.43        | +0.77               | −0.27              | +0.32     | −0.04         |
| Shoot dry weight             | −0.40        | +0.81               | +0.15              | +0.36     | −0.06         |
| Root length                  | −0.34        | +0.64               | −0.54              | +0.08     | −0.19         |
| Shoot length                 | −0.29        | +0.88               | +0.06              | +0.13     | −0.05         |
| Shoot-root ratio, dry-weight | +0.07        | +0.03               | +0.98              | +0.00     | −0.04         |
| Seed weight                  | −0.29        | +0.39               | −0.01              | +0.87     | +0.04         |
| Soil moisture regime         | +0.05        | −0.06               | −0.01              | +0.03     | +0.99         |
| Percent water loss of trays  | −0.93        | +0.31               | −0.09              | +0.09     | −0.00         |
| Factor interpretation        | Water stress | Plant size (Vigour) | Relative root size | Seed size | Soil moisture |

moister and deeper soil layers than smaller plants. These larger plants were more vigorous than the smaller plants; they were not initially larger than the rest. This is so because seed size, determining initial plant size, was extracted separately.

The factors relative root size and soil moisture of the place of seed origin were not important for survival.

The five axes mentioned accounted for 95 percent of total variance in this experiment.

#### Experiment No. 3: — Dry-matter Production under Intermittent Drought Conditions

The correlation analysis (Table I-6) showed that total dry weight after the third drought period was positively correlated with shoot length at the various drought periods, with total dry weight at the beginning of the experiment, with seed weight. Total dry weight and shoot dry weight were negatively correlated with terminal bud percent, i.e. the lighter plants were often those which had stopped growing earlier. The correlations with soil moisture regime were usually negative and not significant.

The principal component analysis (Table I-7) gave loadings for the first four axes and these were interpreted as

being most closely related to needle surface, initial plant size, relative root size, and soil moisture of the place of seed origin.

Needle surface, here suggested by large loadings for shoot length at the three experimental stages, was the most important factor and clearly linked with total dry weight. The role of the assimilating leaf or crown surface in determining growth is well known from silvicultural studies (HAWLEY and SMITH 1954). Initial plant size was next and this factor was again closely related to total dry weight. Relative root size, based upon shoot-root ratio, was in third position and affected total dry weight after the third drought period to a lesser degree.

Soil moisture of the place of seed origin was in the last position and not related to the growth criteria of this experiment.

The combined variance from these four axes was 86 percent of total. The analysis has indicated factors similar to those in Experiment No. 2.

#### Experiment No. 4: — Phenology and Growth

The correlation analysis (Table I-8) showed that there were very highly significant correlations between the date

Table I-6. — Correlation coefficients of Experiment No. 3 based upon 37 single-tree progenies from each of 5 moisture regimes in Populations 4 and 5. Numbers in parentheses refer to measurements in the following stages of the experiment: (0) — before first drought period; (1) — after first drought period; (2) — after second drought period; and (3) — after third drought period.

| Variables                               | x <sub>1</sub> | x <sub>2</sub> | x <sub>3</sub> | x <sub>4</sub> | x <sub>5</sub> | x <sub>6</sub> | x <sub>7</sub> | x <sub>8</sub> | x <sub>9</sub> | x <sub>10</sub> | x <sub>11</sub> | x <sub>12</sub> | x <sub>13</sub> |
|---|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|
| x <sub>1</sub> Total dry weight (3)     | +1.00          | +0.99***       | +0.93***       | +0.05          | −0.34*         | +0.15          | +0.87***       | +0.88***       | +0.85***       | +0.77***        | +0.66***        | +0.71***        | −0.17           |
| x <sub>2</sub> Shoot dry weight (3)     |                | +1.00          | +0.86***       | +0.21          | −0.43**        | +0.10          | +0.89***       | +0.85***       | +0.83***       | +0.73***        | +0.65***        | +0.67***        | −0.20           |
| x <sub>3</sub> Root dry weight (3)      |                |                | +1.00          | −0.30          | −0.13          | +0.26          | +0.74***       | +0.84***       | +0.82***       | +0.78***        | +0.62***        | +0.72***        | −0.10           |
| x <sub>4</sub> Shoot-root ratio (3)     |                |                |                | +1.00          | −0.56***       | −0.35*         | +0.24          | −0.00          | −0.01          | −0.14           | −0.02           | −0.13           | −0.22           |
| x <sub>5</sub> Terminal bud percent (3) |                |                |                |                | +1.00          | +0.26          | −0.61***       | −0.38*         | −0.35*         | −0.01           | −0.16           | +0.01           | +0.09           |
| x <sub>6</sub> Terminal bud percent (2) |                |                |                |                |                | +1.00          | +0.05          | +0.12          | +0.31          | +0.09           | +0.04           | +0.23           | +0.11           |
| x <sub>7</sub> Shoot length (3)         |                |                |                |                |                |                | +1.00          | +0.91***       | +0.86***       | +0.56***        | +0.57***        | +0.44**         | −0.16           |
| x <sub>8</sub> Shoot length (2)         |                |                |                |                |                |                |                | +1.00          | +0.92***       | +0.61***        | +0.53***        | +0.51**         | −0.15           |
| x <sub>9</sub> Shoot length (1)         |                |                |                |                |                |                |                |                | +1.00          | +0.57***        | +0.51**         | +0.46**         | −0.28           |
| x <sub>10</sub> Total dry weight (0)    |                |                |                |                |                |                |                |                |                | +1.00           | +0.83***        | +0.74***        | −0.11           |
| x <sub>11</sub> Shoot length (0)        |                |                |                |                |                |                |                |                |                |                 | +1.00           | +0.56***        | −0.12           |
| x <sub>12</sub> Seed weight             |                |                |                |                |                |                |                |                |                |                 |                 | +1.00           | +0.03           |
| x <sub>13</sub> Soil moisture regime    |                |                |                |                |                |                |                |                |                |                 |                 |                 | +1.00           |

Table 1-7. — Results of the principal component analysis of Ex-

| Axis                      | 1              | 2                  | 3                  | 4             |
|---------------------------|----------------|--------------------|--------------------|---------------|
| Eigenvalue                | 4.68           | 3.49               | 2.03               | 1.07          |
| Percent of total variance | 36             | 27                 | 16                 | 8             |
| Variables                 | Loadings       |                    |                    |               |
| Total dry weight (3)      | +0.76          | —0.60              | +0.08              | —0.07         |
| Shoot dry weight (3)      | +0.76          | —0.57              | +0.21              | —0.09         |
| Root dry weight (3)       | +0.71          | —0.62              | —0.22              | —0.03         |
| Shoot-root ratio (3)      | +0.05          | +0.13              | +0.83              | —0.14         |
| Terminal bud percent (3)  | —0.49          | —0.10              | —0.76              | —0.10         |
| Terminal bud percent (2)  | +0.43          | +0.17              | —0.70              | +0.09         |
| Shoot length (3)          | +0.85          | —0.35              | +0.33              | —0.02         |
| Shoot length (2)          | +0.85          | —0.38              | +0.08              | —0.06         |
| Shoot length (1)          | +0.91          | —0.28              | —0.03              | —0.20         |
| Total dry weight (0)      | +0.33          | —0.89              | —0.09              | —0.08         |
| Shoot length (0)          | +0.28          | —0.81              | +0.08              | —0.09         |
| Seed weight               | +0.34          | —0.75              | —0.17              | +0.11         |
| Soil moisture regime      | —0.12          | +0.01              | —0.11              | +0.98         |
| Factor interpretation     | Needle surface | Initial plant size | Relative root size | Soil moisture |

of the last spring frost and growth initiation, day length and growth cessation, and degree-days and height growth. Most of the climatic variables were correlated with each other as well as with latitude and longitude. Growth initiation, growth cessation, and height growth were also correlated with soil moisture regime, but only 6 percent of total variance could be explained by this factor as indicated by the coefficient of determination.

The principal component analysis (Table 1-9) suggested that the first three axes were most closely related to the factors day length, temperature, and seed size.

Day length ranked first because of the large loadings for day length, longitude and latitude. The physiological responses consistently coupled with this factor were growth cessation and duration of growth. That growth cessation largely depends upon day length is in agreement with the findings of other investigators (WAREING 1956).

Temperature, or temperature timing, was next as shown by the loadings for date of last spring frost, lengths of growing and frost-free seasons, and degree-days. The best tie here was with growth initiation. The close relationship between growth initiation in the spring and temperature has been reported from many species (PAULEY and PERRY 1954, RUBNER 1960).

Seed size was last and related to prolepsis and height growth.

The total variance associated with the first three axes amounted to 85 percent. Another 5 percent were contributed by the fourth non-rotated axis which was related to soil moisture of the place of seed origin.

### General Discussion

The physiological response and morphological characters of black spruce in experiments dealing with germination, drought resistance, growth and phenology were well correlated with population characteristics such as seed weight, with the test environment, and with native habitats. Prin-

Table 1-8. — Correlation coefficients of Experiment No. 4 based upon 121 single-tree progenies in Populations 1 to 9.

| Variables   | x1   | x2       | x3       | x4       | x5       | x6       | x7       | x8      | x9       | x10      | x11      | x12      | x13      | x14      | x15      | x16      |
|---|------|----------|----------|----------|----------|----------|----------|---------|----------|----------|----------|----------|----------|----------|----------|----------|
| x1 Prolepsis, 1st year  | 1.00 | +0.52*** | +0.26**  | +0.39*** | +0.40*** | +0.48*** | +0.33*** | +0.07   | —0.42*** | —0.32*** | —0.41*** | +0.48*** | +0.35*** | +0.48*** | —0.36*** | —0.45*** |
| x2 Growth cessation, 1st year                                   |      | 1.00     | +0.79*** | +0.92*** | +0.88*** | +0.88*** | +0.20*   | +0.21*  | —0.93*** | —0.71*** | —0.75*** | +0.88*** | +0.73*** | +0.86*** | —0.86*** | —0.94*** |
| x3 Growth initiation, 2nd year                                  |      |          | 1.00     | +0.75*** | +0.64*** | +0.65*** | +0.08    | +0.23*  | —0.74*** | —0.53*** | —0.72*** | +0.78*** | +0.71*** | +0.75*** | —0.73*** | —0.72*** |
| x4 Growth cessation, 2nd year                                   |      |          |          | 1.00     | +0.99*** | +0.88*** | +0.05    | +0.26** | —0.98*** | —0.79*** | —0.62*** | +0.81*** | +0.62*** | +0.84*** | —0.94*** | —0.96*** |
| x5 Duration of growth, 2nd year                                 |      |          |          |          | 1.00     | +0.87*** | +0.04    | +0.25*  | —0.96*** | —0.80*** | —0.56*** | +0.76*** | +0.56*** | +0.80*** | —0.92*** | —0.95*** |
| x6 Height growth until 2nd year                                 |      |          |          |          |          | +1.00    | +0.26**  | +0.22*  | —0.88*** | —0.76*** | —0.55*** | +0.73*** | +0.54*** | +0.75*** | —0.83*** | —0.88*** |
| x7 Seed weight  |      |          |          |          |          |          | +1.00    | —0.09   | —0.12    | —0.05    | —0.26**  | +0.26**  | +0.22*   | +0.21*   | —0.02    | —0.16    |
| x8 Soil moisture regime   |      |          |          |          |          |          |          | +1.00   | +0.26**  | —0.18*   | —0.22*   | +0.24**  | +0.23*   | +0.21*   | —0.23*   | —0.26**  |
| x9 Latitude N   |      |          |          |          |          |          |          |         | +1.00    | +0.79*** | +0.87*** | —0.84*** | —0.68*** | —0.86*** | +0.95*** | +0.99*** |
| x10 Longitude W   |      |          |          |          |          |          |          |         |          | +1.00    | +0.22*   | —0.46*** | —0.32*** | —0.44*** | +0.79*** | +0.78*** |
| x11 Mean date of last spring frost                              |      |          |          |          |          |          |          |         |          |          | +1.00    | —0.94*** | —0.96*** | —0.84*** | +0.59*** | +0.66*** |
| x12 Length of growing season (days above +6° C)                 |      |          |          |          |          |          |          |         |          |          |          | +1.00    | +0.90*** | +0.95*** | —0.73*** | —0.84*** |
| x13 Length of frost-free season                                 |      |          |          |          |          |          |          |         |          |          |          |          | +1.00    | +0.75*** | —0.62*** | —0.63*** |
| x14 Degree-days above +6° C                                     |      |          |          |          |          |          |          |         |          |          |          |          |          | +1.00    | —0.77*** | —0.86*** |
| x15 Day length June 21st  |      |          |          |          |          |          |          |         |          |          |          |          |          |          | +1.00    | +0.90*** |
| x16 Weekly reduction of day length, June 21st to September 21st |      |          |          |          |          |          |          |         |          |          |          |          |          |          |          | +1.00    |

**Table 1-9. — Results of the principal component analysis of Experiment No. 4. Axis 4 is from the earlier standard analysis, i. e. before varimax rotation.**

| Axis  | 1          | 2           | 3         | 4             |
|---|------------|-------------|-----------|---------------|
| Eigenvalue  | 7.08       | 5.05        | 1.38      | 0.86          |
| Percent of total variance                                   | 44         | 32          | 9         | 5             |
| Variables   | Loadings   |             |           |               |
| Prolepsis, 1st year   | +0.36      | —0.26       | +0.59     | +0.31         |
| Growth cessation, 1st year                                  | +0.77      | —0.57       | +0.14     | —0.04         |
| Growth initiation, 2nd year                                 | +0.52      | —0.67       | —0.09     | —0.15         |
| Growth cessation, 2nd year                                  | +0.88      | —0.44       | —0.03     | —0.04         |
| Duration of growth, 2nd year                                | +0.90      | —0.36       | —0.02     | —0.02         |
| Height growth until 2nd year                                | +0.84      | —0.35       | +0.20     | +0.09         |
| Seed weight   | —0.01      | —0.20       | +0.82     | +0.24         |
| Soil moisture regime  | +0.16      | —0.32       | —0.46     | +0.81         |
| Latitude N  | —0.86      | +0.49       | —0.02     | +0.03         |
| Longitude W   | —0.93      | —0.00       | —0.00     | —0.04         |
| Mean date of last spring frost                              | —0.23      | +0.95       | —0.13     | +0.07         |
| Length of growing season (days above +6° C)                 | +0.49      | —0.85       | +0.15     | —0.04         |
| Length of frost-free season                                 | +0.27      | —0.90       | +0.07     | —0.08         |
| Degree-days above +6° C                                     | +0.56      | —0.74       | +0.14     | —0.07         |
| Day length June 21st  | —0.86      | +0.41       | +0.06     | +0.08         |
| Weekly reduction of day length, June 21st to September 21st | —0.86      | +0.47       | —0.07     | —0.00         |
| Factor interpretation                                       | Day length | Temperature | Seed size | Soil moisture |

principal component analysis proved useful in extracting the most important factors from the numerous correlations.

Several properties of principal component analysis must be considered before evaluating the results. It is clear that component analysis explores only the linear relationships, depends upon the method of sampling, the sample size, and the accuracy of experiments. The variance associated with each axis in this analysis made it possible to rank the proposed underlying factors, but the factors found may depend upon the variables initially chosen for measurement in each experiment (WALLIS 1965). The eigenvalues and variances contributed by each axis are therefore comparable mainly within the same experiment and analysis, constituting a relative scale to measure the effect of the proposed underlying factors in that analysis. In a final evaluation of the main factors, i. e. those of greatest relevance to the hypotheses being tested, it is therefore best to consider both the contribution to total variance and the loadings for morphological and physiological variables (Tables 1-10).

Soil moisture of origin ranked last in all four experiments. The axes most closely related to this factor, i. e. the fourth or fifth axis in each analysis, contributed from 5 to 10 percent of total variance. The loadings for physiological and morphological variables were small and ranged from 0.02 to 0.31. This is a factor of minor importance.

This result is confirmed by evidence from other sources. It is obvious that adaptation to soil moisture and the development of drought resistance are not isolated processes, but depend upon a number of interacting factors in the total environment. Intense selection of drought resistant genotypes would probably take place in the year of ger-

mination when soil moisture is most critical (PLACE 1955, EIS 1965), but at that stage seed size and seedling vigour are still major factors. As the germination experiment indicated, seedlings of seed from dry and fresh sites were more vigorous than those from moist and wet sites, possibly due to the greater availability of nitrogen at drier locations discussed by WEETMAN (1962). Some moist and wet sites, on the other hand, may develop drought conditions because trees growing there have a shallow root system and may not reach the water table lowered in a dry season (SHIRLEY 1934, DENYER and RILEY 1964). As a re-

**Table 1-10. — Summary of main factors showing contributions to total variance, and range of loadings for morphological and physiological variables. Loadings are given in absolute values, i. e. neglecting signs.**

| Factor             | Experiment No. | Related axis | Variance percent | Range of loadings |
|--------------------|----------------|--------------|------------------|-------------------|
| Soil moisture      | 1              | 5            | 8                | 0.12—0.25         |
|                    | 2              | 5            | 10               | 0.04—0.19         |
|                    | 3              | 4            | 8                | 0.02—0.20         |
|                    | 4              | 4            | 5                | 0.02—0.31         |
| Relative root size | 2              | 3            | 14               | 0.06—0.54         |
|                    | 3              | 3            | 16               | 0.03—0.75         |
| Seed size          | 1              | 1            | 25               | 0.00—0.85         |
|                    | 2              | 4            | 11               | 0.00—0.36         |
|                    | 4              | 3            | 9                | 0.02—0.59         |
| Vigour             | 1              | 2            | 19               | 0.13—0.85         |
| Plant size         | 2              | 2            | 29               | 0.03—0.88         |
| Needle surface     | 3              | 1            | 36               | 0.05—0.91         |
| Geographic origin  | 1              | 3            | 16               | 0.04—0.27         |
| Day length         | 4              | 1            | 76               | 0.26—0.90         |
| Temperature        | 4              | 2            |                  |                   |



sult, dry and wet sites may differ less than is usually expected. Even if drought resistant genotypes are selected on dry upland sites, populations must be sufficiently isolated to accumulate gene differences (FORD 1964). None of the stands tested for drought resistance in this study were isolated. In other species where drought resistance was related to local soil conditions, isolation was either partially or completely developed (HABECK 1958, BRIX 1959, FERRELL and WOODARD 1966). However, most studies dealing with drought resistance in forest trees show that this had developed in response to regional climates (MEULI and SHIRLEY 1937, KRIEBEL 1963, KRAL 1965, PHARIS and FERRELL 1966).

Relative root size ranked next in importance. The third axes in Experiments No. 2 and 3, to which this factor was related, contributed 14 and 16 percent of total variance. Loadings ranged from 0.03 to 0.76 but were rather low for the survival and growth criteria of main interest. Therefore the influence of this factor was rather limited.

Seed size varied in importance. It was most closely related to the first axis in Experiment No. 1, the fourth axis in Experiment No. 2, and the third axis in Experiment No. 4, contributing 25, 11, and 9 percent of total variance, respectively. The loadings of physiological and morphological variables ranged from 0.00 to 0.85. This was a very important factor initially, but its role declined from the time of germination to the completion of the second growing season. This trend is well recorded in the literature (BALDWIN 1942).

Several similar factors constitute the following group: vigour, second axis of Experiment No. 1, contributing 19 percent of total variance; plant size, second axis of Experiment No. 2, contributing 29 percent; and needle surface, first axis of Experiment No. 3, contributing 36 percent. Loadings for physiological and morphological variables ranged from 0.03 to 0.91. These factors carry considerable weight, particularly in early growth and survival. They were more important than root size and much more important than soil moisture of origin.

A significant position was occupied by geographic origin. In Experiment No. 1 this factor was represented by the third axis, accounting for 16 percent of total variance; in Experiment No. 4 by the first and second, including the effects of day length and temperature, with 76 percent of total variance. The loadings for morphological and physiological variables ranged from 0.04 to 0.90. Thus the role of geographic origin was relatively small during germination and early growth, but it increased greatly during the two-year period and dominated in the nursery stage.

In conclusion, the analyses show that black spruce development from seed to two-year-old seedling is influenced by the following factors: Seed size and germinant seedling vigour dominate during the earliest period. Subsequent drought resistance and development is a function of plant size, vigour, and particularly duration of growth which is controlled by regional climates.

Because natural selection would tend to favour genotypes best adapted to regional climates which are more consistent from year to year than are local climates and soil moisture, a clinal variation pattern should develop. The results of this study therefore support the first hypothesis, namely, that variation is essentially clinal in response to continuous ecological factors. The problem will be further investigated in Part II of this paper.

## Summary

1. *Picea mariana* is a species adaptable to various soils and climates with a transcontinental range from Alaska to Pennsylvania. To test the two hypotheses that variation in this species is either clinal, or clinal and ecotypic, seed from 9 forest regions, 24 stands, and 148 single trees was collected between 42 and 60 degrees of N. latitude and 77 and 112 degrees of W. longitude. Experiment No. 1 dealt with germination and early development, Experiment No. 2 with survival of seedlings during their first growing season under continuous drought conditions, Experiment No. 3 with first-season growth under intermittent drought conditions, and Experiment No. 4 with phenology and growth to the end of the second growing season. Measurements were evaluated by correlation and principal component analyses.

2. The principal component analysis based on correlations of all measured variables extracted uncorrelated factors related to characteristics of the population, of the test environment, and of the native habitat. Given in order of importance they were:

- for germination and early development: seed size, vigour, geographic origin, competition, and soil moisture of the place of seed origin;
- for survival under continuous drought conditions: water stress, plant size, seed size, relative root size, and soil moisture of origin;
- for growth under intermittent drought conditions: needle surface, initial plant size, relative root size, and soil moisture of origin;
- for phenology and growth: day length of origin, temperature of origin, seed size, and soil moisture of origin.

3. In general, root size and soil moisture played a minor role because they were only weakly related to physiological variables such as germination rate, survival index, and growth. The importance of seed size declined over the two-year testing period while that of geographic origin, including day length and temperature, increased.

4. The results support the first hypothesis that variation is essentially clinal. There is little evidence for ecotypic variation.

## Résumé

Titre de l'article: Variation *génétique* des semis de *Picea mariana*. I. Corrélations avec les facteurs écologiques.

1. *Picea mariana* représente une espèce ligneuse qui s'adapte facilement aux différentes conditions édaphiques et climatiques de son aire transcontinentale qui s'étend de l'Alaska à la Pennsylvanie.

Dans le but de vérifier les deux hypothèses, à savoir que la variation au sein de cette espèce peut être soit clinale, soit clinale et ecotypique, un certain nombre de semences furent récoltées dans 9 régions forestières, groupant 24 peuplements et 148 individus, situés entre les 42<sup>e</sup> et 60<sup>e</sup> degrés de latitude nord et entre 77<sup>e</sup> et 112<sup>e</sup> degrés de longitude ouest.

La première expérience consista en une étude sur la germination et les premiers stades de développement; la deuxième expérience tint compte de la survivance des semis durant la première saison de croissance sous des conditions de sécheresse constante; à la troisième expérience, des semis d'une première saison de croissance furent soumis à des conditions intermittentes de sécheresse; finalement la quatrième expérience traita de la phénologie et de la croissance jusqu'à la fin de la seconde saison de végétation. Les mesures furent calculées par corrélation et l'analyse des composantes principales.

2. L'analyse des composantes principales s'appuyant sur les corrélations établies entre toutes les variables numériques, mis en relief les facteurs non-correlatifs se rapportant aux caractéristiques de la population, l'environnement de l'expérience, et de l'habitat naturel.

Suivant un ordre d'importance, nous avons utilisé les critères suivant pour:

- la germination et les premiers *stades de développement*: la dimension de la semence et la vigueur du semis, l'origine géographique, la compétition, l'humidité du sol du lieu d'origine de la semence.
- la survivance des plantules sous des conditions de sécheresse continue: la tension hydrique, les dimensions de la plantule et de la semence, la dimension relative des racines et l'humidité du sol au lieu d'origine.
- la croissance sous des conditions intermittentes de sécheresse: la surface des aiguilles, la dimension initiale des plantules, la dimension relative des racines et l'humidité du sol au lieu d'origine.
- la *phénologie* et la croissance: la photoperiode et la température du lieu d'origine, la dimension des semences et l'humidité du sol au lieu d'origine.

3. D'une façon générale, le critère de la dimension des racines ainsi que celui de l'humidité du sol jouèrent un rôle de moindre importance parce qu'ils furent faiblement en relation avec les variables physiologiques telles que le taux de germination, l'index de la survivance et la croissance.

L'importance du facteur de la dimension des graines diminua à mesure que la période de deux ans suivant laquelle s'effectuèrent les observations avançait, contrairement aux facteurs de l'origine géographiques incluant la température et la photoperiode, qui prirent une plus grande importance durant cette même période de temps.

4. Les résultats obtenus se rallient à la première hypothèse, à savoir que la variation est essentiellement clinale. Il y a peu d'évidence pour variation ecotypique.

### Literature Cited

ANDERSSON, E.: Cone and seed studies in Norway spruce (*Picea abies* [L.] KARST.). *Studia Forestalia Suecica* 23, 214 p. + App. (1965). — BAKUZIS, E. V., and HANSEN, H. L.: Balsam Fir *Abies balsamea* (LINNAEUS) MILLER. A monographic review. Univ. of Minnesota Press, Minneapolis 1965, 445 p. — BALDWIN, H. I.: Forest tree seed of the north temperature regions with special reference to North America. *Chronica Botanica Co., Waltham, Mass.*, 1942, 240 p. — BARTELS, H.: Über die Abhängigkeit der Keimlingsgröße vom Gewicht und vom Entwicklungszustand des Samens bei Fichte und Kiefer. *Allg. Forst- u. Jagdztg.* 127: 164–168 (1956). — BARTLETT, M. S.: Some examples of statistical methods of research in agriculture and applied biology. *J. Roy. Stat. Soc., Suppl.*, 4: 137–170 (1937). — BASHAM, J. T., and MORAWSKI, J. R.: Cull studies, the defects and associated basidiomycete fungi in the heartwood of living trees in the forests of Ontario. *Dept. Forestry Publ.* 1072, 69 p. (1964). — BESLEY, L.: Relationship between wood fibre properties and paper quality. *Pulp and Paper Res. Inst. Canada Tech. Rept.* 156, 19 p. (1959). — BRIX, H.: Some aspects of drought resistance in loblolly pine seedlings. Dissertation, Agricultural and Mechanical College of Texas. Summary, p. 83–85 (1959). — BRYAN, M. S.: Interglacial pollen spectra from Greenland. *Danmarks Geol. Unders.* II, Raekke, No. 80, 65–72 (1954). — BURGAR, R. J.: The effect of seed size on germination, survival and initial growth in white spruce. *For. Chron.* 40: 93–97 (1964). — BURLEY, J.: Variation in seed characteristics of Sitka spruce. *Advanc. Front. Plant Sci.* (New Delhi) 10: 11–24 (1964). — CASSIE, R. M.: Multivariate analysis in the interpretation of numerical plankton data. *N. Z. J. Sci.* 6: 36–39 (1963). — DAMMAN, A. W. H.: Some forest types of central Newfoundland and their relation to environmental factors. *For. Sci. Monogr.* 8, 62 p. (1964). — DENYER, W. B., and RILEY, C. G.: Drought effects on young spruce stands in poor sites. *For. Chron.* 40: 206–208 (1964). — DEPT. OF MINES AND TECHNICAL SURVEYS: Atlas of Canada. Queen's Printer, Ottawa, 110 plates (1957). — DORF, E.: Climatic changes of the past and present. *Am. Scient.* 48: 341–364 (1960). — EIS, S.: Development of white spruce and alpine fir seedlings on cut-over areas in the central Interior of British Columbia. *For. Chron.* 41: 419–431 (1965). — FERRELL, W. K., and WOODARD, E. S.: Effects of seed origin on drought resistance of Douglas-fir (*Pseudotsuga menziesii* [MIRB.] FRANCO). *Ecology* 47: 499–503 (1966). — FORD, E. B.: Ecological genetics. Wiley, New York, 335 p. (1964). — FRUCHTER, B.: Introduction to factor analysis. Van Nostrand, Princeton, N. J., 280 p. (1954). — HABECK, J. R.: White cedar ecotypes in Wisconsin. *Ecology* 39: 457–463 (1958). — HARMAN, H. H.: Modern factor analysis. Univ. of Chicago Press, Chicago, 471 p. (1960). — HAWLEY, R. C., and SMITH, D. M.: The practice of silviculture. 6th ed. Wiley, New York,

525 p. (1954). — HEER, O.: Flora fossilis arctica. Bd. 1. Schulthess, Zürich, 192 p., plates (1868). — HEINSELMAN, M. L.: Silvical characteristics of black spruce (*Picea mariana*). U.S. Forest Serv., Lake States For. Exp. Sta., Sta. Paper 45, 30 p. (1957). — HEINSELMAN, M. L.: Forest sites, bog processes, and peatland types in the glacial Lake Agassiz region. Minnesota. *Ecol. Monogr.* 33: 327–374 (1963). — HOLST, M.: Seed selection and tree breeding in Canada. Canada Dept. Forestry, For. Res. Br. Tech. Note 115, 20 p. (1962). — HORTON, K. W., and LEES, J. C.: Black spruce in the foothills of Alberta. Canada Dept. Forestry, For. Res. Br. Tech. Note 110, 54 p. (1961). — HOTELLING, H.: Simplified calculation of principal components. *Psychometrika* 1: 27–35 (1936). — KENDALL, M. G.: A course in multivariate analysis. Griffin, London, 185 p. (1957). — KRAL, F.: Physiologische Frühstestversuche an Herkünften der Grünen Douglasie. *Zentralbl. ges. Forstw.* 82: 129–149 (1965). — KRAUS, B. S., and CHOI, S. C.: A factorial analysis of the prenatal growth of the human skeleton. *Growth* 22: 231–242 (1958). — KRIEBEL, H. B.: Selection for drought resistance in sugar maple. *World Cons. For. Genet. and For. Tree Improvmt., Stockholm*, 1963. *FAO/FORGEN* 63-3/9, 5 p. (1963). — LAMOTTE, R. S.: Catalogue of the Cenozoic plants of North America through 1950. *Geol. Soc. Am. Mem.* 51: 1–381 (1952). — LANGNER, W.: Keimungsverlauf bei den Herkünften eines Provenienzversuches mit *Larix leptolepis* (SIEB. et ZUCC) GORD. *Silvae Genet.* 9: 165–167 (1960). — LINTEAU, A.: Forest site classification of the northeastern coniferous section Boreal forest region, Quebec. Canada Dept. North. Aff. and Nat. Resources, For. Br. Bull. 118, 85 p. (1955). — LOUCKS, O. L.: A forest classification for the Maritime Provinces. *Proc. Nova Scotian Inst. Sci.* 25: 85–167 (1959–60). — LOWRY, G. L.: Forest soil-site studies. I. Objectives, sampling, preliminary results on black spruce. *Pulp and Paper Res. Inst. Canada Tech. Rep.* 368, 23 p. (1964). — LUTZ, H. J., and CHANDLER, R. F.: Forest soils. Wiley, New York, 514 p. (1946). — MACGILLIVRAY, H. G.: Report on forest genetic studies at Acadia Forest Experiment Station, 1955. In: *Proc. Fourth Meetg. Comm. For. Tree Breedg. Canada*, 1956. Maple, Ont. Appendix L: 1–9 (1956). — MACLEAN, D. W., and BEDELL, G. H. D.: Northern Clay Belt growth and yield survey. Canada Dept. North. Aff. and Nat. Resources, For. Br., Res. Div. Tech. Note 20, 31 p. (1955). — MEULI, L. J., and SHIRLEY, H. L.: The effect of seed origin on drought resistance of green ash in the prairie-plains states. *J. Forestry* 35: 1060–1062 (1937). — MILLAR, J. B.: The silvicultural characteristics of black spruce in the Clay Belt of northern Ontario. Master's thesis, Fac. Forestry Univ. Toronto, 81 p. (1936). — MORGENTERN, E. K.: Genetische Variation von *Picea mariana* (MILL.). B. S. P. Dissertation, Math.-Naturw. Fak. Univ. Hamburg, 77 p. (1966). — ONTARIO DEPT. OF LANDS AND FORESTS: Manual of seed collecting. *Pub. TM-513*, 28 p. (1963). — PAULEY, S. S., and PERRY, T. O.: Ecotypic variation of the photoperiodic response in *Populus*. *J. Arnold Arbor.* 35: 167–188 (1954). — PEARCE, S. C.: *Biological statistics: an introduction.* McGraw-Hill, New York, 212 p. (1965). — PHARIS, R. P., and FERRELL, W. D.: Differences in drought resistance between coastal and inland sources of Douglas fir. *Canad. J. Bot.* 44: 1661–1669 (1966). — PLACE, I. C. M.: The influence of seed-bed conditions on the regeneration of spruce and balsam fir. Canada Dept. North. Aff. and Nat. Resources, For. Br. Bull. 117, 87 p. (1955). — RIGTER, F. I.: Pinus: the relationship of seed size and seedling size to inherent vigour. *J. For.* 43: 131–137 (1945). — ROHMDDER, E.: Die Bedeutung der Keimschnelligkeit bei forstlichen Samenarten. *Proc. Int. Seed Test. Assoc.* 27: 657–671 (1962). — ROWE, J. S.: Forest regions of Canada. Canada Dept. North. Aff. and Nat. Resources, For. Br. Bull. 123, 71 p. (1959). — RUBNER, K.: Die pflanzengeographischen Grundlagen des Waldbaues. 5th ed. Neumann, Radebeul, 630 p. (1960). — SCHELL, G.: Keimschnelligkeit als Erbeigenschaft. *Silvae Genet.* 9: 48–53 (1960). — SEAL, H. L.: Multivariate statistical analysis for biologists. Methuen, London, 207 p. (1964). — SHIRLEY, H. L.: Observation on drought injury in Minnesota forests. *Ecology* 15: 42–48 (1934). — SNEDECOR, G. W.: *Statistical methods.* 5th ed. Iowa State College Press, Ames, Iowa, 534 p. (1956). — STEEL, R. G. D., and TORRIE, J. H.: Principles and procedures of statistics. McGraw-Hill, New York, 481 p. (1960). — U. S. DEPT. OF AGRICULTURE: Map of the forest regions of the United States. U. S. Dept. Agric. Pub. M-5154 (1948). — U. S. DEPT. OF AGRICULTURE: *Silvics of forest trees of the United States.* Agriculture Handbook 271, 762 p. (1965). — VAN GROENENWOUD, H.: Ordination and classification of Swiss and Canadian coniferous forests by various biometric and other methods. *Ber. geobot. Inst. ETH, Stifg. Rübel, Zürich*, 36, 28–102 (1965). — VINCENT, A. B.: Black spruce. A review of its silvics, ecology and silviculture. Canada Dept. Forestry Pub. 1100, 79 p. (1965). — VISHER, S. S.: Climatic Atlas of the United States. Harvard University Press, Cambridge, Mass., 403 p. (1954). — WALLIS, J. R.: Multivariate statistical methods in hydrology — a comparison using data of known functional relationship. *Water Resources Rev.* 1: 447–461 (1965). — WARREING, P. F.: Photoperiodism in woody plants.

Ann. Rev. Plant Physiol. 7: 191–214 (1956). — WEETMAN, G. F.: Nitrogen relations in a black spruce (*Picea mariana* MILL.) stand subject to various fertilizer and soil treatments. Pulp and Paper Res. Inst. Canada. Woodl. Res. Index No. 129, 112 p. (1962). — WHITE, P. A.: The computation of eigenvalues and eigenvectors of a matrix. J. Soc. Industr. Appl. Math. 6: 393–437 (1958). — WILLIAMSON, M. H.: An ecological survey of a Scottish herring fishery. Part IV: Changes in the plankton during the period 1949 to 1959. Bull. Mar. Ecol. 5: 208–229 (1961). — WITHROW, R. B., (ed.): Photo-

periodism and related phenomena in plants and animals. Am. Assoc. Adv. Sci. Pub. 55, 903 p. (1959). — WRIGHT, J. W., and BULL, W. I.: Geographic variation in European black pine — two-year results. For. Sci. 8: 32–42 (1962). — YEATMAN, C. W.: Geographic variation in jack pine (*Pinus banksiana* LAMB.) seedlings. Ph. D. thesis, Yale Univ., 231 p. (1966a). — YEATMAN, C. W.: Germinant size of jack pine in relation to seed size and geographic origin. In: U. S. For. Serv. Res. Paper NC-6: 28–36 (1966b). — YOUNGBERG, C. T.: Effect of soil fertility on the physical and chemical properties of tree seed. J. For. 50: 880–882 (1952).

# Genetic Variation in Seedlings of *Picea mariana* (Mill.) BSP.

## II. Variation Patterns

By E. K. MORGENSTERN<sup>1)</sup>

(Received for publication May 10, 1968)

### Introduction

The genetic processes of mutation, recombination, migration, isolation and selection change the frequency of genes in natural populations and lead to the development of certain variation patterns. For each species this pattern is complex in origin but may be explained in a simplified manner by assuming that natural selection is the dominant genetic process. By making this assumption, the pattern can be related to environmental factors, and it is then possible to define the factors which are most important. This approach has been taken in Part I of this paper (MORGENSTERN 1969).

The combined effect of all genetic processes must be measured in a different manner. The expected subdivision of the species can be investigated by means of hierarchical sampling and analysis of variance. Thus the total variance can be divided into the components associated with populations (among regions), subpopulations (among stands within regions), half-sib families (among single-tree progenies within stands), and within half-sib families (among replications of the same single-tree progenies). The study was continued in this way and the results are given in the present paper.

### Methods

#### Experiments

The four experiments carried out in this study dealt with germination and early development, survival and dry-matter production under drought conditions, and growth and phenology. Details of the experimental procedure were outlined elsewhere (MORGENSTERN 1966, 1969). Thirteen physiological and morphological characters are considered in this paper to test certain black spruce populations for the presence of a clinal or clinal and ecotypic variation pattern.

### Criteria for Classification

Clinal variation (HUXLEY 1938) is defined as a gradation in measurable characters of organisms. It is thought that

this type of variation may develop after an initial disruptive selection (MATHER 1953) in the base population migrating from the centre of origin of a species, which is then followed by stabilizing selection and gene exchange among adjacent populations over the species range (HALDANE 1948, FISHER 1950).

Ecotypic variation (TURESSON 1922) is marked by an abrupt pattern change resulting from disruptive selection within populations where habitats are discontinuous and from stabilizing selection within subpopulations. Therefore, both clinal and ecotypic variation are similar in that they are forms of adaptive genetic variation, but they differ in the underlying evolutionary mechanisms. Thus their distinction is important (STERN 1964). Furthermore, it must be recognized that subpopulation differences may also arise in non-adaptive fashion by migration from different refugia (BOUVAREL 1939); random drift in small populations (WRIGHT and BULL 1963), and inbreeding (MAYR 1963).

The variation pattern found in this study will be classified in the following manner: If the population component of variance is larger than the subpopulation and family component, variation will be considered clinal. If the subpopulation component is larger than the population and family component, variation will be considered ecotypic regardless of the genetic processes involved. Direct comparisons of the variance components can also be made provided that a similar sampling system has been used (STERN 1964, SQUILLACE 1966).

### Statistical Analysis

The statistical analysis was based upon the model

$$Y_{ijkl} = \mu + p_i + s_{ij} + f_{ijk} + w_{ijkl}$$

where  $Y_{ijkl}$  represents the plot mean of a particular character measured in replication  $l$  of the  $k$ -th family in the  $j$ -th subpopulation and the  $i$ -th population;  $\mu$  the overall mean of the experiment;  $p_i$ ,  $s_{ij}$ , and  $f_{ijk}$  the effects of the  $i$ -th population,  $j$ -th subpopulation in population  $i$ , and  $k$ -th half-sib family in the  $j$ -th subpopulation of population  $i$ , respectively; and  $w_{ijkl}$  the within-family deviation including error and the effect of replication  $l$ . Although randomized block and modified lattice square designs were used in the physical layout of experiments, the preceding model assumes a completely randomized structure. This simplified the presentation of results and the calculation of mean square coefficients, without resulting in large disadvantages. All experimental units were considered ran-

<sup>1)</sup> Research Scientist, Department of Fisheries and Forestry, Petawawa Forest Experiment Station, Chalk River, Ontario, Canada. Second paper based upon a dissertation for the degree of Dr. rer. nat. submitted in 1966 to the Mathematisch-Naturwissenschaftliche Fakultät, Universität Hamburg, Germany. The larger part of the experimental work was performed at Institut für Forstgenetik und Forstpflanzenzüchtung, Bundesforschungsanstalt für Forst- und Holzwirtschaft, Schmalenbeck, Germany.